Urinary cotinine measurement in patients with Buerger's disease—Effects of active and passive smoking on the disease process

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Although Buerger's disease is known to be closely related to smoking, no objective analysis of the smoke-associated problems has been performed. In this study, cotinine, the major metabolite of nicotine, was used as a sensitive marker to measure levels of active smoking and the exposure of nonsmokers to tobacco smoke because it has a relatively long half-life and because cotinine levels can be determined by noninvasive means in urine. According to urinary cotinine levels, 40 patients with Buerger's disease were classified as (1) smokers: those with urinary cotinine levels above 50 ng/mg creatinine; (2) passive smokers: those with levels between 10 and 50 ng/mg creatinine; and (3) nonsmokers who did not experience noticeable passive smoking: those with levels below 10 ng/mg creatinine. There were 10 smokers, 9 passive smokers, and 21 nonsmokers. The course of the disease, after the initial treatment at our hospital, was studied retrospectively. Seven of the 10 smokers, none of the 9 passive smokers, and 4 of the 21 nonsmokers experienced aggravation of the disease. Of the four nonsmokers who experienced aggravation, three had still been smokers and one had been exposed to tobacco smoke in the workplace at the time of relapse. There was a significant difference in the aggravation rate between the smokers' group and the other two groups. Among the smokers, the seven patients whose conditions worsened showed significantly higher cotinine levels than the three remaining patients who were in the stage of remission. The conclusions were: (1) a very close relation between active smoking and the course of Buerger's disease was established, and (2) effects of passive smoking on the disease process were still inconclusive. (J VASC SURG 1991;14:53-8.)

Buerger's disease is characterized by peripheral arterial occlusion of the extremities most frequently in young adult male smokers.[1,2] In general, all patients with Buerger's disease have a history of smoking, and smoking is also known to be closely related to exacerbations of the disease.[1,3] The outlook in regard to the effects on the limbs of a patient with Buerger's disease is favorable if he stops smoking, but the disease gets progressively worse if he continues to smoke.[3,4]

However, we have occasionally found that the disease recurred in patients who stated that they had abstained from smoking. Many of them may have been lying about their smoking habits: some were deemed to have denied themselves the pleasure of smoking but had been exposed to tobacco smoke in the home and workplace. Because there is no objective test to evaluate smoking, previous studies have had to depend on patients' testimony of smoking habits. An objective method of evaluation of the degree of active and passive smoking is necessary to elucidate the relationship between smoking and Buerger's disease.

By measuring urinary concentration of cotinine, the major metabolite of nicotine, we found a correlation between smoking and the natural course of Buerger's disease in retrospective study.

PATIENTS AND METHODS

Urine samples were collected for measurement of nicotine and cotinine levels from 50 volunteers (23 smokers and 27 nonsmokers) without noticeable passive smoking and whose statements of smoking histories were regarded as reliable. The time pattern of nicotine and cotinine excretion was studied to judge whether alkaloid is suitable as the marker for

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smoking from the standpoint of the half-life of cotinine in the human body. For this purpose, urinary nicotine and cotinine levels of a healthy nonsmoker (one of the authors) were measured after he had smoked one cigarette and after he had been placed in a passive smoking environment. For our passive smoking experiment, the subject was placed in an airtight room (19.1 m^3) and exposed to side-stream tobacco smoke from a total of 40 cigarettes for 3 hours.

Urine samples from 40 patients with Buerger's disease were collected (one for each case) when the patients came to our clinic. Each patient's statement about current smoking status and involuntary exposure to smoking was requested at each visit. Our clinical criteria for the diagnosis of Buerger's disease are: (1) history of smoking; (2) onset before the age of 50 years; (3) infrapopliteal arterial occlusive disease; (4) either upper-limb involvement or phlebitis migrans; and (5) absence of atherosclerotic risk factors other than smoking. The clinical diagnosis of Buerger's disease was made when all five requirements were met.[1,3] Infrapopliteal obstruction was confirmed by arteriography in each case, and arteriographic findings such as tapering or abrupt occlusion, corkscrew or rootlike appearance of collaterals, and corrugated appearance served as supporting evidence. All of the patients had a history of smoking before the onset of the disease. At onset, the age of these 40 patients ranged from 26 to 49 years (mean, 37 years). There were 38 men and 2 women. All 40 patients had been treated in our institution for more than 1 year, and their case histories were reviewed retrospectively. The initial treatments of these patients were bypass grafting and sympathetectomy in 2, bypass grafting in 4, sympathetectomy in 24, and medical treatment only in 10. The follow-up period ranged from 1 to 22 years, with a mean of 8.3 years. In case of recurrence of pain at rest, ischemic ulceration, or graft failure (except early failure, less than 30 days), which were confirmed by follow-up surveillance, the patient was considered clinically to have "aggravation of the disease."

Urinary nicotine and cotinine levels were determined by high-performance liquid chromatography (HPLC) according to Mizobuchi's method[5] with some modifications. We changed the extraction procedures in order to assess very low levels of these alkaloids. Urine samples were stored at −20° C until analysis. Ten milliliters of urine was centrifuged. After the addition of 4 gm sodium chloride, 0.1 ml 25% ammonium hydroxide, and 2 ml chloroform, the urine samples were shaken for 10 minutes and centrifuged at 12,000 rpm for 10 minutes. The chloroform layer was collected and then shaken with 5 ml of 0.1 N hydrochloric acid for 10 minutes and centrifuged at 12,000 rpm for 10 minutes. The resulting aqueous layer was shaken with 2 gm sodium hydrochloride, 0.2 ml ammonium hydrochloride, and 1 ml chloroform, and then centrifuged at 12,000 rpm. Fifty microliters of this chloroform layer was used for the HPLC. Average total recoveries were 98% for nicotine and 85% for cotinine. The detection limits of nicotine and cotinine were 2 ng/ml and 3 ng/ml, respectively. Urinary nicotine and cotinine values were normalized by creatinine excretion and expressed as nanograms per milligram of creatinine.

Statistical significance was assessed by Student's t test or chi-square analysis, and the results were considered significant at $p < 0.05$.

RESULTS

For the healthy control subjects, urinary nicotine levels were 576 ± 474 ng/mg creatinine (mean value ± standard deviation) in the smokers, and 5.2 ± 3.8 ng/mg creatinine in the nonsmokers who did not have perceptible involuntary exposure to tobacco smoke ($p < 0.01$). Urinary cotinine levels for these two groups were also significantly different (859 ± 814 ng/mg creatinine in the smokers vs 5.6 ± 2.3 ng/mg creatinine in the nonsmokers, $p < 0.01$). Urinary cotinine levels discriminated between the smokers and the nonsmokers more distinctly than nicotine levels. Therefore those with urinary cotinine levels above 50 ng/mg creatinine may be regarded as smokers (Fig. 1). In smokers, urinary excretion of cotinine roughly correlated to self-reported cigarette consumption (Fig. 2). Fig. 3 shows urinary nicotine and cotinine levels in a healthy nonsmoker after he had smoked one cigarette and after he had been exposed to side-stream smoke. Urinary cotinine elevation after active smoking lasted for 60 hours. The urinary cotinine level after passive smoking was lower compared with the level after active smoking, but it showed the same rise and fall as the level after active smoking. The disappearance of nicotine from the urine was faster than that of cotinine. Because of this, only the urinary cotinine level was used for studies on the patients.

Fig. 4 shows the urinary cotinine levels in patients with Buerger's disease. All three patients who confessed themselves to be current smokers had cotinine levels that were higher than 50 ng/mg creatinine. Of the 37 patients who asserted that they were not active smokers, seven (19%) had cotinine levels above 50 ng/mg creatinine. According to our definition, these

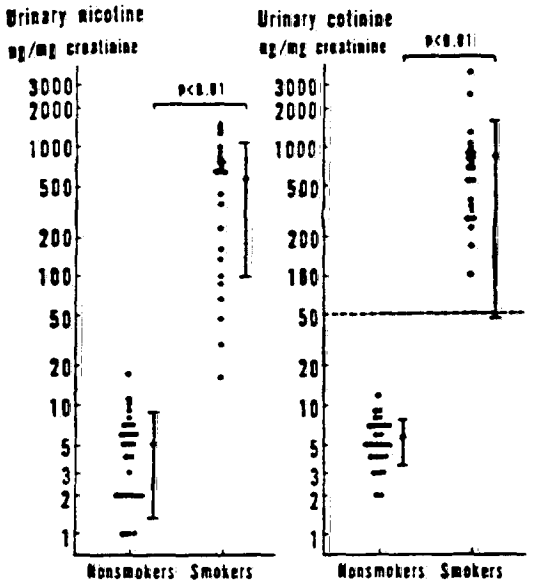

Fig. 1. Urinary nicotine levels *(left)* and cotinine levels *(right)* of nonsmokers and smokers, in healthy control subjects. There were significant differences between the two groups ($p < 0.01$). Cotinine levels discriminate between smokers and nonsmokers more distinctly than nicotine levels do.

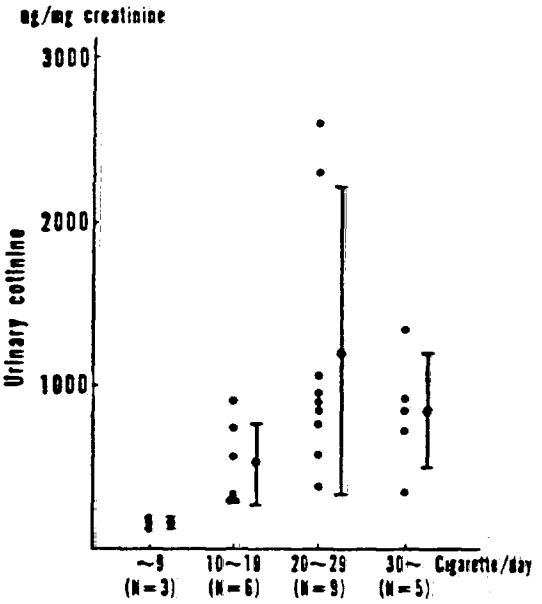

Fig. 2. Urinary cotinine levels in smokers. Smokers were classified into four groups on the basis of self-reported cigarette consumption. Urinary cotinine levels roughly correlated to daily cigarette consumption.

seven patients were considered active smokers, whereas the other 30 patients were regarded as exsmokers. The 30 exsmokers were then divided into two groups, on the grounds of self-reported involuntary exposure to smoking. Urinary cotinine levels were 10.2 ± 4.2 ng/mg creatinine in those who were involuntarily exposed to smoking and 6.1 ± 3.5 ng/mg creatinine in those who were not exposed ($p < 0.01$) (Fig. 5). On the basis of these results, we decided that for this study, those with urinary cotinine levels between 10 and 50 ng/mg creatinine would be identified as nonsmokers with noticeable passive smoking (passive smokers) and those with levels below 10 ng/mg creatinine would be identified as nonsmokers without perceptible passive smoking (Fig. 5).

The 40 patients were classified into three groups: (1) those with urinary cotinine levels above 50 ng/mg creatinine (active smokers), (2) those with cotinine levels between 10 and 50 ng/mg creatinine (passive smokers), and (3) those with cotinine levels below 10 ng/mg creatinine (nonsmokers without noticeable passive smoking). Eventually, 10 patients were classified as active smokers, 9 as passive smokers, and 21 as nonsmokers. The disease worsened in 7 (70%) of the 10 smokers; in none (0%) of the 9 passive smokers; and in 4 (19%) of the 21 nonsmokers. There were significant differences in the rate of the aggravation of the disease between the smokers and the passive smokers ($p < 0.01$) and between the smokers and the nonsmokers ($p < 0.01$). However, no significant differences in the rate of aggravation were found between the passive smokers and the nonsmokers (Fig. 6). Of the four exsmokers who experienced worsening of the disease, three admitted that they had still been active smokers at that time. The other one stated that he had been involuntarily exposed to noticeable smoking in the workplace all day at the time of recurrence. This patient had sympathetectomy and bypass operation of the left leg for the initial treatment. Four years later, femorocrural bypass grafting in the right leg was necessary because of right popliteal artery occlusion that was a result of a skip lesion. Thereafter, however, he has kept away from tobacco smoke in the workplace and he has been doing well for 2 years (Fig. 6). Among the 10 current smokers, the mean cotinine level for the seven patients who had aggravation of the disease was significantly higher than the level for the three patients who did not experience relapses (1208 ± 734 ng/mg creatinine vs 147 ± 79 ng/mg creatinine, $p < 0.05$).

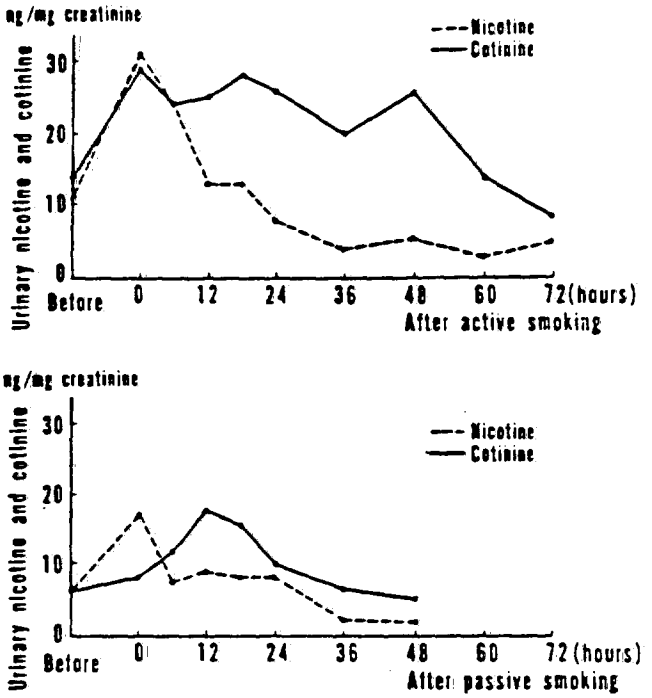

Fig. 3. Urinary nicotine *(broken line)* and cotinine *(solid line)* excretion over time. After active smoking *(above)*, and after high involuntary exposure to smoke *(below)*, in a healthy nonsmoker. Cotinine levels decreased more slowly than nicotine levels did.

DISCUSSION

Carboxyhemoglobin or nicotine concentrations have been used as indicators of smoking.[6,7] In vascular surgery, carboxyhemoglobin has been used to determine smoking habits of patients who had arterial reconstructive operations,[8,9] and Wiseman et al.[9] reported that the median concentration of carboxyhemoglobin was significantly higher in those patients whose grafts had failed than in those whose grafts were patent. However, blood carboxyhemoglobin concentrations have not proved to be markers specific to smoking, and nicotine measurements have been regarded as providing more accurate assessments.[10] Recently, cotinine has been considered a more sensitive marker of smoking because it has a much longer plasma half-life than nicotine does (about 30 hours vs about 30 minutes).[11,12] In this study, urinary cotinine levels clearly discriminated between smokers and nonsmokers. By measurement of cotinine levels, 10 patients were identified as active smokers, although seven of them claimed to have quit smoking. Of these 10 active smokers, seven experienced aggravation, and there was a significant difference in the rate of aggravation between active smokers and exsmokers. It was confirmed that active smoking was very closely related to recurrences of Buerger's disease. Three former smokers, however, experienced worsening of the disease even though their urinary cotinine level remained within a nonsmoker's or a passive smoker's range. Since the urinary cotinine elevation after smoking lasted for only 60 hours, our assessments of smoking were limited to a very short period. Past smoking habits cannot be estimated by ill-timed measurement of cotinine, a short-term marker, when patients have abstained from smoking. Serial examination of urinary cotinine levels should be performed to solve this problem.

Because the number of cigarettes smoked roughly correlated with the urinary cotinine level,[13,14] this level may reflect the intensity of smoking. However, there was considerable variation in cotinine excretion among subjects who smoked approximately the same number of cigarettes. These variations were assumed to be caused by differences in nicotine content per cigarette and in the manner of smoking (inhaling or puffing, frequency, length of cigarettes smoked).[13,14] In this study, among the patients who continued to smoke, those who experienced aggravation of the disease had significantly higher cotinine levels than

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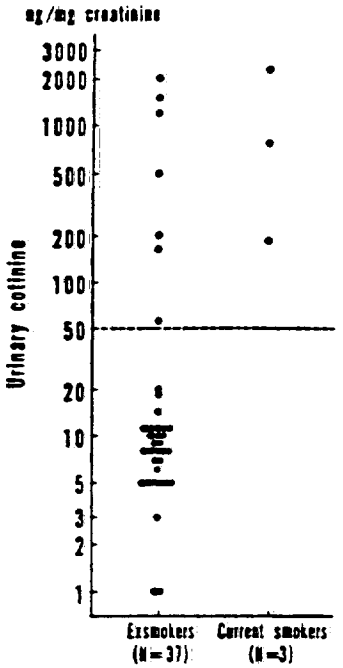

Fig. 4. Urinary cotinine levels. Patients were divided into two groups according to their statements about their smoking habits. Regardless of their claims, they were classified as smokers if they had urinary cotinine levels above 50 ng/mg creatinine.

those who were in remission. However, even among those who experienced aggravation, the urinary cotinine levels varied widely. It seems impossible to predict which patient will become worse, judging from the number of cigarettes that were smoked.

Recent studies have indicated that involuntary exposure to smoking may be as harmful as active smoking.[15] Sinzinger and Kefalides[16] reported that passive smoking reduced platelet sensitivity to anti-aggregatory prostaglandins (E_1, I_2, D_2), and the reduction in sensitivity was much more severe in nonsmokers than in smokers. Passive smoking might exert a poor influence on the cardiovascular system for nonsmokers. In this study, the influence of involuntary exposure to smoking on Buerger's disease was studied by measurement of urinary cotinine levels, but no significant relationship between involuntary exposure to smoking and recurrence of the disease was found. However, there was one patient who had aggravation of the disease, who testified to have abandoned smoking habits, and this person had the urinary cotinine level of a nonsmoker. Because he had been involuntarily exposed to noticeable smoking at the time of worsening of the disease and is not

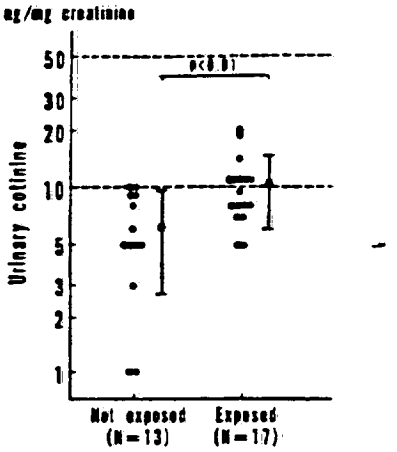

Fig. 5. Urinary cotinine levels of nonsmokers without involuntary exposure to smoking and nonsmokers with involuntary exposure to smoking. There was a significant difference between the two groups ($p < 0.01$).

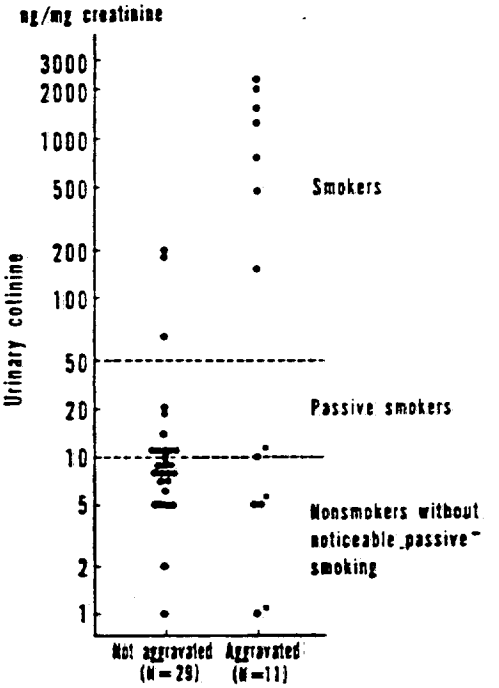

Fig. 6. Urinary cotinine levels and the course of Buerger's disease. There were significant differences in aggravation between the smokers' group and the other two groups, but no significant differences were found between passive smokers and nonsmokers without noticeable passive smoking. Three *(asterisks)* of the four nonsmokers with aggravated conditions stated that they had been smoking at the time of worsening of the disease.

exposed to tobacco smoke now, the worsening of his disease may be associated with the past involuntary exposure to smoking. The incorrect timing of urinary cotinine measurement may explain why no significant relationship was found between passive smoking and the worsening of the disease in this study. A cooperative epidemiologic and clinical study that is based on the long-term and timely evaluation of effects on health of involuntary exposure to smoking may provide the evidence to support the hypothesis that passive smoking can influence the occurrence of Buerger's disease and the worsening of the disease process.

In conclusion, cotinine is a sensitive but short-term marker of smoking. Smoking tobacco was very closely related to the course of Buerger's disease, but no significant correlation between passive smoking and the disease process has been found yet.

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